Docket No.: 0230-0222PUS1

nventor: Ichiro HIRAO et al.

· Fitle: NUCLEOSIDE OR NUCLEOTIDES HAVING NOVEL

UNNATURAL BASES AND USE THEREOF

REPLACEMENT SHEET

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Figure 1

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Figure 2

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Figure 3

bis(dimethylamino)naphthalene,(CH3O)3PO,0°C,2h. (2)(n-Bu3NH)2P2O,0°C,10min (c)(1)POCl3,(CH3O)3PO,0°C,2h. (2)(n-Bu3NH)2P2O,0°C,10min. (d)(1)POCl3,1,8-(a)I2,KI,Na2CO3,100°C,4h. (b)CF3CONHCH2CCH,Pd(Ph3P),Cul,Et3N,DMF,rt,4-6h. (3)conc.NH,OH,rt,10h. A
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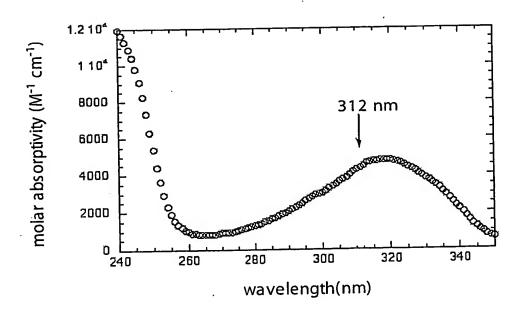
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Figure 4

b)



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Figure 5

a)

5'-end primer; 39-mer

39.45

:5'-GGTAATACGACTCACTATAGGGAGTGGAGGAATTCATCG

3'-end primer; 29-mer

29.45

:5'-GCAGAAGCTTGCTGTCGCTAAGGCATATG

29.45s84

 $: 5 \ '- \texttt{GCAGAAGCTTGCTGTC} \underline{\texttt{s}} \texttt{CTAAGGCATATG}$

29.45s87

:5'-GCAGAAGCTTGCTSTCGCTAAGGCATATG

29. 45s92

:5'-GCAGAAGCsTGCTGTCGCTAAGGCATATG

29.45s84/92 :5'-GCAGAAGCsTGCTGTCsCTAAGGCATATG

b)

5'-GGGAGUGGAG GAAUUCAUCG AGGCAUAUGU CGACUCCGUC UUCCUUCAAA CCAGUUAUAA AUUGGUUUUA GCAUAUGCCU UAGCGACAGC AAGCUUCUGC

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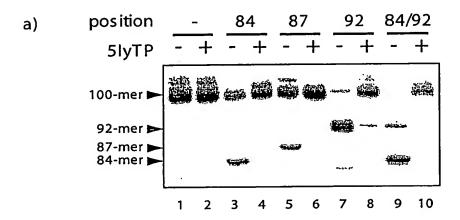
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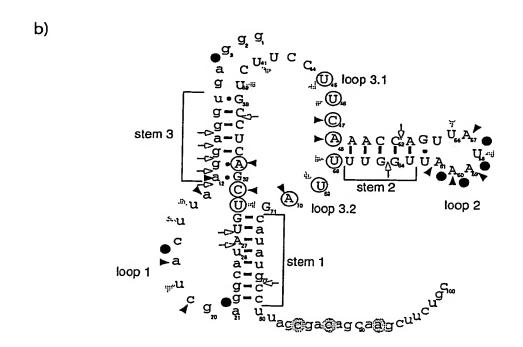
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Figure 6





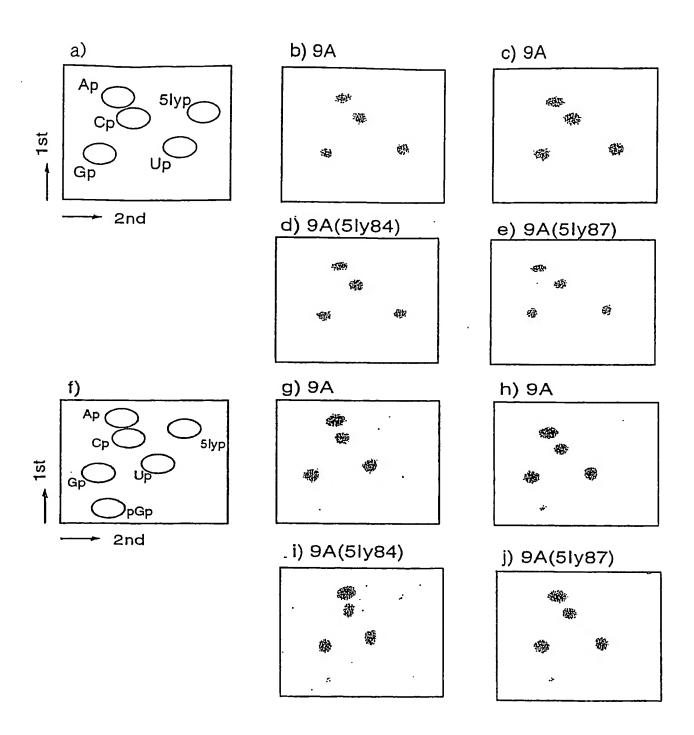
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Figure 7



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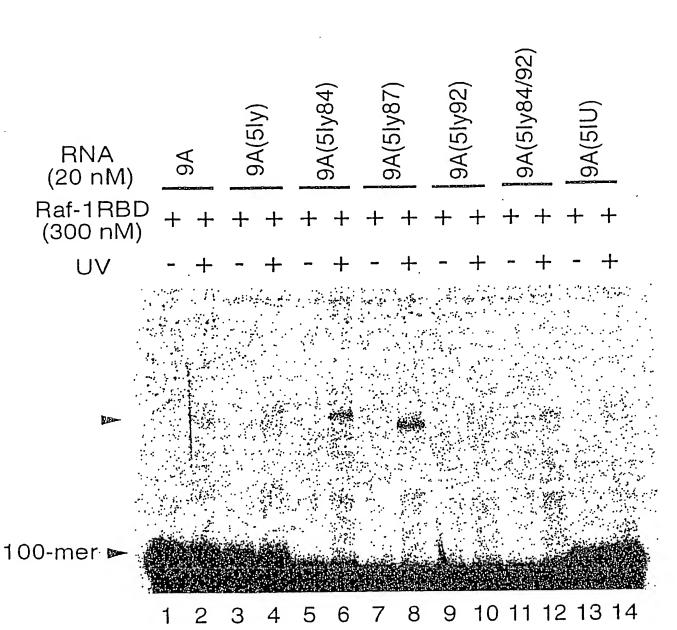
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Figure 8



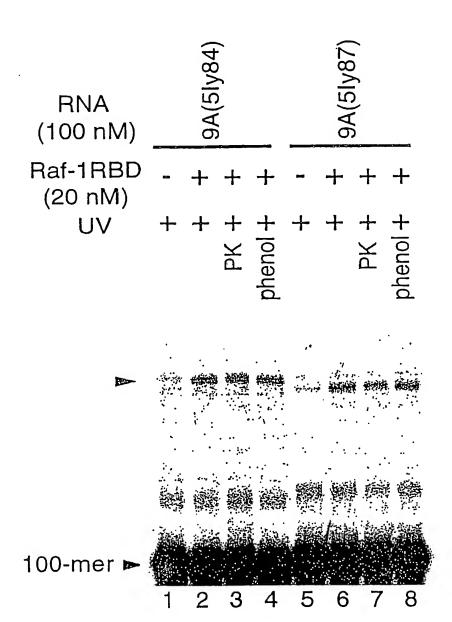
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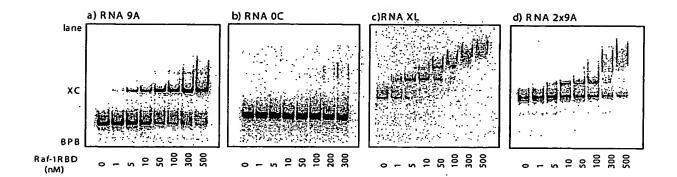
Figure 9



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Figure 10



e) RNA 9A :100-mer

5 - GGGAGUGGAGGAAUUCAUCGAGGCAU [-N45-] CAUAUGCCUUAGCGACAGCUUCUGC-3 '

AUGUCGACUCCGUCUUCCAAACCAGUUAUAAAUUGGUUUUAG

RNA 9A(5ly87):100-mer

5'-GGGAGUGGAGGAAUUCAUCGAGGCAU[-N45-]cauaugccuuagcga5IyCAGCAAGCUUCUGC-3'

RNA 2x9A:200-mer

5 '-GGGAGUGGAGGAAUUCAUCGAGGCAU [-N45-] CAUAUGCCUUAGCGACAGCAUCCUCC-

 $\hbox{-}GGGAGUGGAGGAAUUCAUCGAGGCAU\,[\,\hbox{-N}_{45}\,\hbox{-}\,]\,CAUAUGCCUUAGCGACAGCAUCUGC}\,\hbox{-}\,3\,\hbox{'}$

RNA 0C:100-mer

 $\verb|5|'-GGGAGUGGAGGAAUUCAUCGAGGCAU[-N45-]| CAUAUGCCUUAGCGACAGCUUCUGC-3|'$

CUGGGAACCCUAUCUUGCUUUUGGUAGCUGUAUUCACCUGUAACAG

RNA XL: cross-linking product generated from two molecules of 9A(5ly87)

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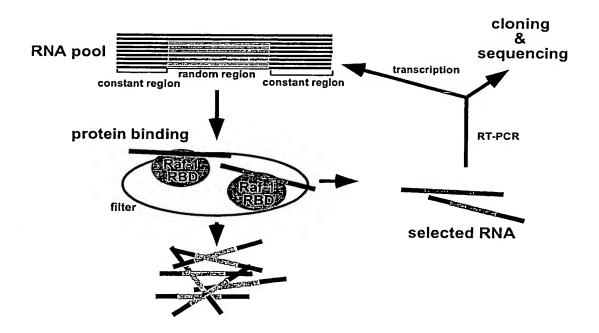
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Figure 11

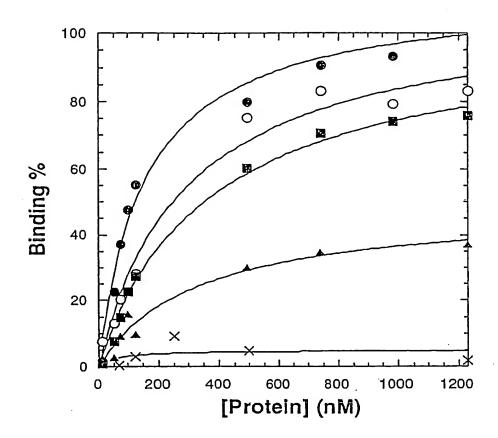


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Figure 12



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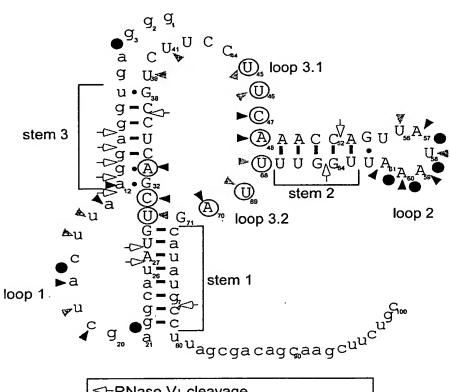
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Figure 13



- <->¬RNase Vı cleavage
- Mung Bean Nuclease cleavage
- CMCT modification
- DMS modification
- notection from chemical modification by the Raf-1 GST-RBD

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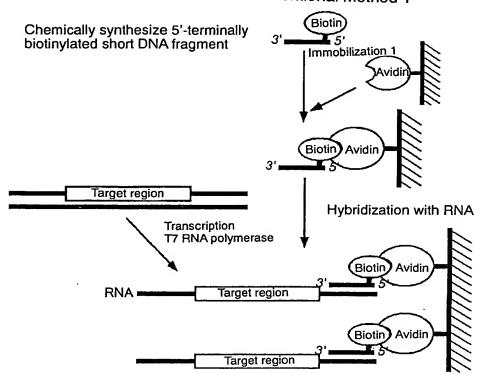
Inventor: Ichiro HIRAO et al.

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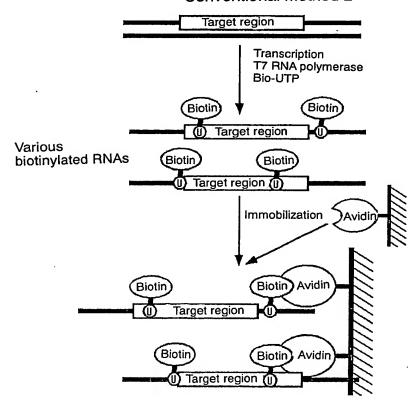
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Figure 14

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Conventional Method 2



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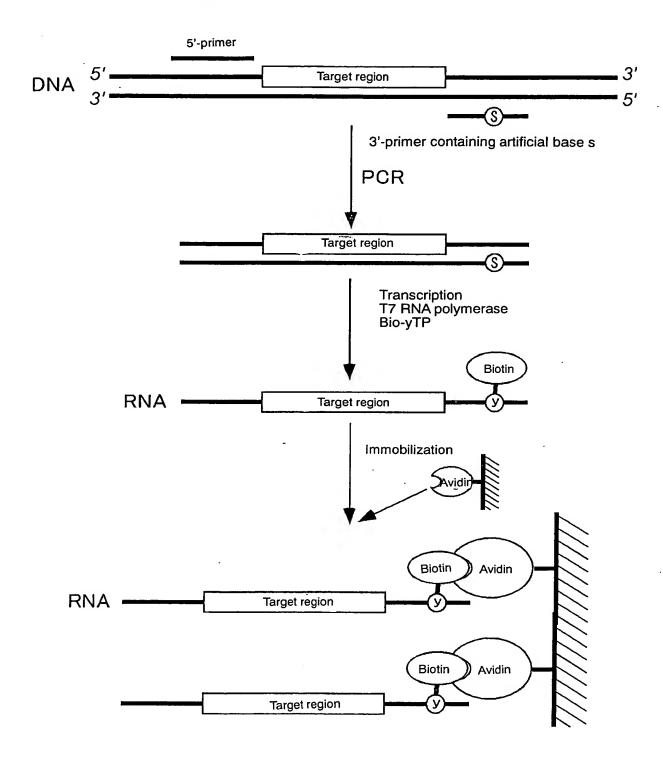
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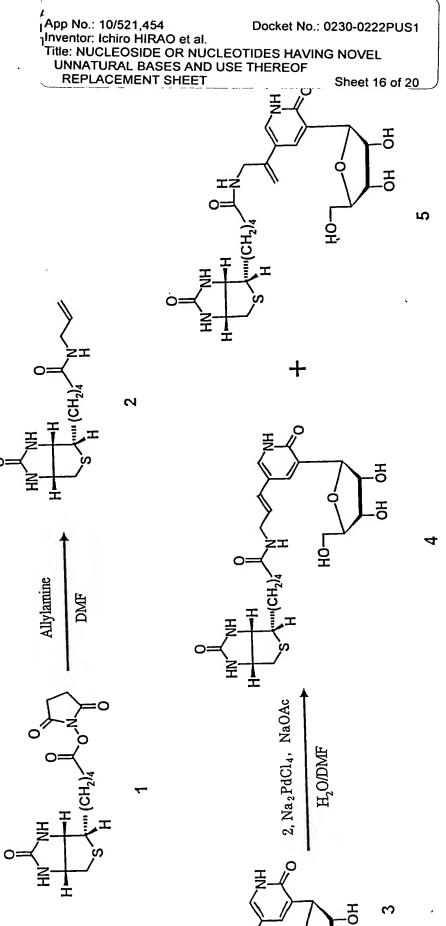
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Figure 14 (Continued) REPLACEMENT SHEET

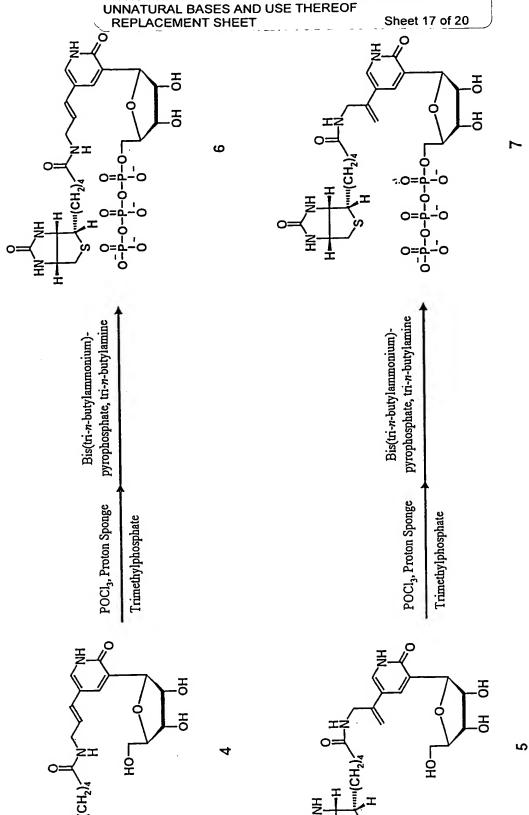
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Inventive Method based on artificial base pairing





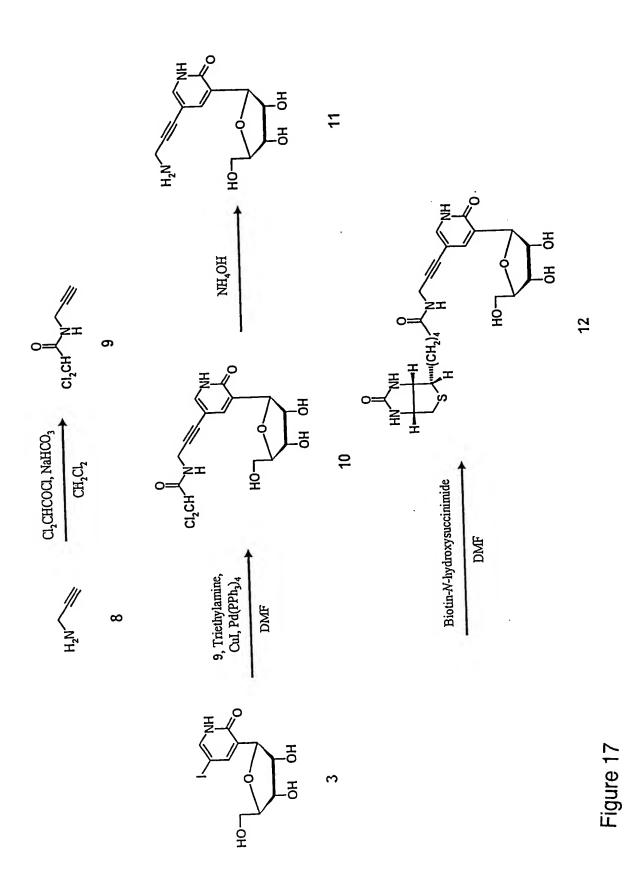
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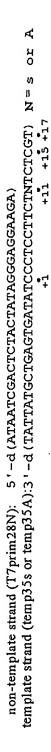
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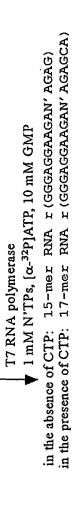
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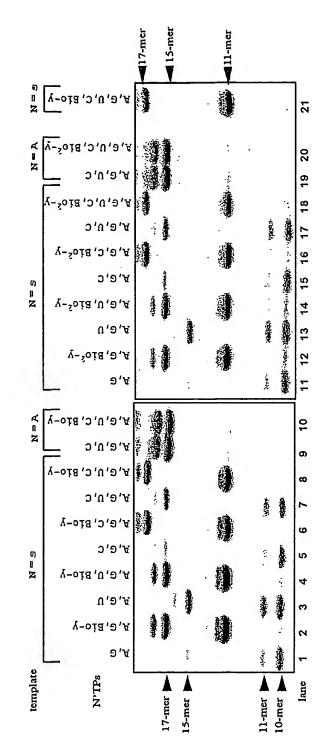
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AIRNA containing normal nucleotides and Bio-y or Bio2-y ► RNA containing only normal nucleotides

Figure 19